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Chromosomal characterization of the Japanese dormouse *Glirulus japonicus* Schinz (Rodentia, Muscardinidae)

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Abstract. The chromosome complement of Japanese dormouse, *Glirulus japonicus*, was investigated by Q-, C-, and Ag-NOR-stainings. The diploid number of this species is 2n=46 with the arm number (FN) of 88. C-bands were located at the centromeric regions of all chromosome pairs. The Y chromosome did not stain intensely in C-staining, indicating no positive C-band in both arms. Nucleolus organizer regions appeared in the telomeric regions of all pairs of chromosomes 8, 10, and 11, which were confirmed by *in situ* hybridization for rRNA genes.

Keywords : *Glirulus japonicus*, Q-band, C-band, NORs

Introduction

The Japanese dormouse, *Glirulus japonicus* belongs to Rodentia, Muscardinidae and is the only extant species that represents this genus. This species is endemic to Honshu, Shikoku, and Kyushu islands of Japan and known as one of natural monuments in Japan because of a single species in genus *Glirulus* and its limited distribution (Nishimura, 1996). Recently, Suzuki *et al.* (1997) compared sequences of mitochondrial 12S rRNA gene of Japanese dormouse with those of the forest dormouse (*Dryomys nitedula*) and the common dormouse (*Muscardinus avellanarius*), both of which are thought to be closely related to the genus *Glirulus*, and described that the sequences from Japanese dormouse were distinct from any sequences of the latter two species and that the extent of the differences was somewhat similar to that between the rat (*Rattus norvegicus*) and the hamster (*Mesocricetus auratus*). Moreover, Suzuki *et al.* (1997) stated that the Japanese dormouse belongs to its own subfamily, Glirulinae, and can be subdivided into at least two genetically different groups. Therefore, further study including cytogenetics holds promise of yielding better understanding of the phylogeny of this species. The present communication provides the first report of the banded karyotypes and chromosomal localization of rRNA genes of the Japanese dormouse.

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Materials and methods

Chromosome preparations of a male and two female Japanese dormice captured in Daibosatsu Pass in Yamanashi Prefecture, Japan were made from the primary fibroblast cultures of skin tissues by our routine air-drying method. Q- and C-banding were carried out according to Yoshida *et al.* (1975) and Sumner (1972), respectively. Silver staining of nucleolus organizer regions (Ag-NORs) was made using the method of Howell and Black (1980).

For localization of ribosomal RNA (rRNA) genes, fluorescence *in situ* hybridization (FISH) was performed according to the method previously described (Oshida and Yoshida, 1996), using HG125 and HG126 plasmid DNA of human 18S and 28S rRNA genes as a probe.

Results and discussion

The diploid chromosome number of the Japanese dormouse was 46 with the fundamental number (FN) of 88, as described previously (Tsuchiya, 1979). The karyotype consisted of 12 pairs of metacentrics, 6 pairs of submetacentrics, 4 pairs of subtelocentrics, a submetacentric X chromosome, and the smallest metacentric Y chromosome (Fig. 1a). All chromosomes are distinctive with Q-banding, as shown in Fig. 1b. As noted, the euchromatic region of the X chromosome

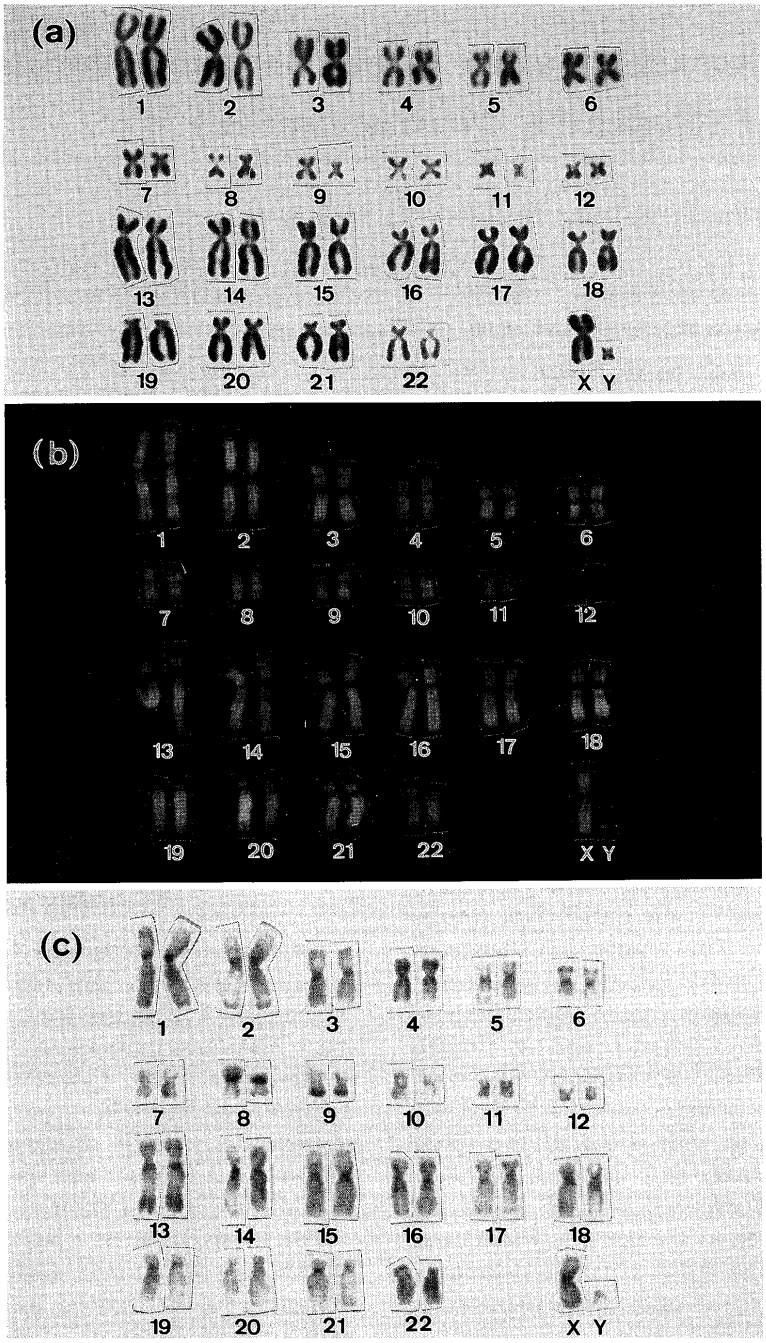

Figure 1. Karyotypes of a male Japanese dormouse *Grilulus japonicus*, a : conventional, b : Q-banded, c : C-banded karyotype.

exhibited the two major bands, as characteristic of therian mammals (Pathak and Stock, 1975). The short arm of the Y chromosome was negatively stained in Q-banding, and its long arm stained positively.

Centromeric regions of all chromosomes were heterochromatic (Fig. 1c). Both arms of the Y chromosome were not stained positively, indicating no heterochromatin. C-band negative Y chromosomes are known in several mammalian species ; ribbon seal *Phoca fasciata* (Arnason, 1977), Arctic ground squirrel *Citellus parryi* (Lyapunova *et al*., 1980), giant white-tailed rat *Uromys caudimacuulatus* (Baverstock *et al*., 1982), Afgan pika *Ochotona rufescens* (Kimura *et al*., 1983), roe deer *Capreolus capreolus* (Rubini and Fontana, 1988), and red and white giant flying squirrel *Petaurista alborufus* and red giant flying squirrel *Petaurista petaurista* (Oshida *et al*., 1992). Therefore, the C-band negative Y is one of characteristics in the

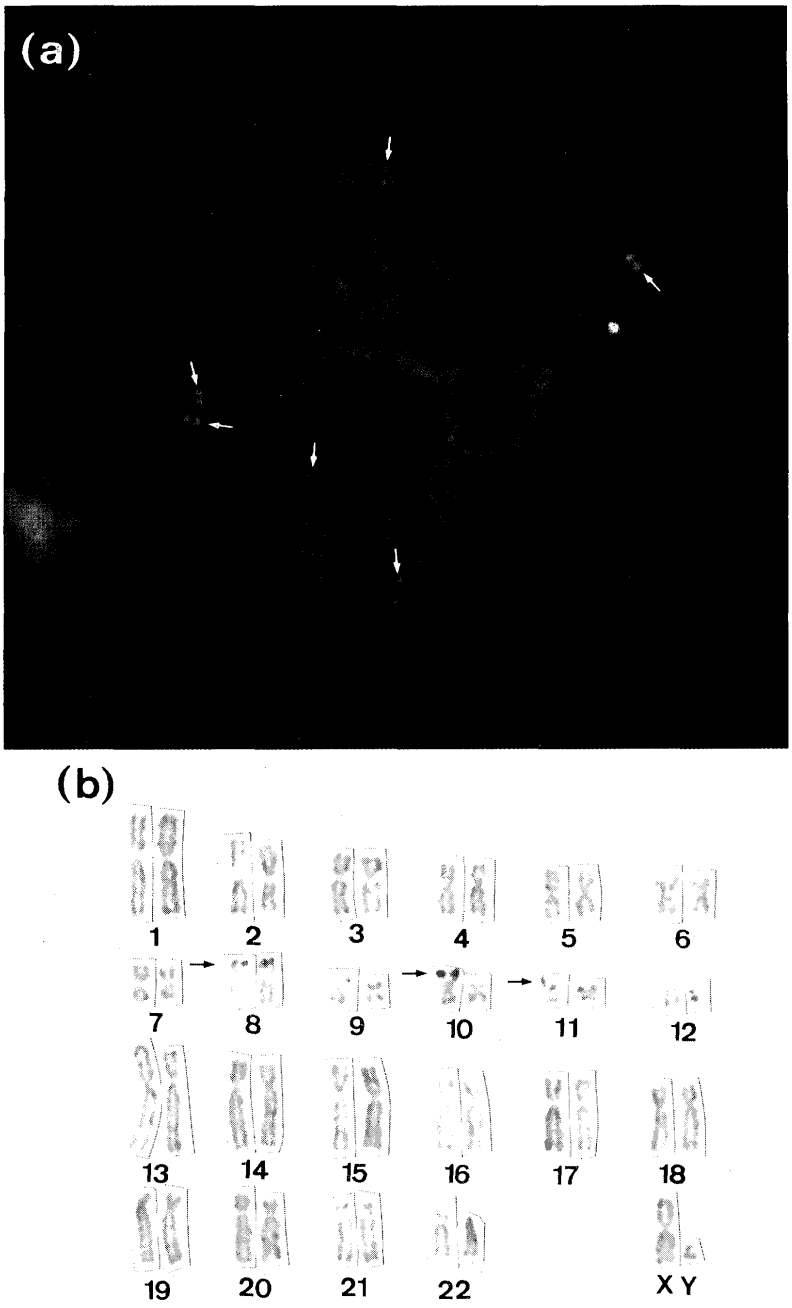

Figure 2. Localization of NORs in a male Japanese dormouse chromosomes, a : after FISH with rRNA genes, b : silver staining. Each arrow indicates sites of NORs.

karyotype of Japanese dormouse, although we analyzed the Y chromosome in only a male specimen.

Hybridization signals by FISH for rRNA genes were detected in the telomeric regions of the short arms of all pairs of chromosomes 8, 10, and 11 (Fig. 2). However, silver staining revealed that the number of Ag-NOR bearing chromosomes varied in metaphases to metaphases, from four to five chromosomes, being polymorphic, although FISH signals appeared constantly in six chromosomes in all metaphases examined. These results suggest that only active NORs were positively stained after Ag-staining in this species as reported previously (Miller *et al*., 1976a, b ; Suzuki *et al*., 1992). Thus, it appears that *in situ* hybridization is the most effective tool available for localizing rRNA genes, because of the variation in the number of Ag-NOR bearing chromosomes possibly due to intercellular variability.

As far as we know, cytogenetic studies of the related genera of *Dryomys* and *Muscardinus* have not been reported. Although much remains to be determined their karyotypes, comparative analysis of karyotypic data is likely to need for establishing phylogenetic relationships and tentative pathways of their karyotypic evolution.

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